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Cytotoxic and Schistosomidal Activities of Extract, Fractions and Isolated Compounds from *Zanthoxylum Leprieurii* (Rutaceae)

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Abstract

Schistosomiasis is a major and chronic neglected tropical disease. The existing treatment does not kill immature schistosomes and have serious adverse side effect. It is well known that some parasites are responsible for causing specific cancers in humans including bladder cancer from *Schistosoma haematobium* infection. So, novel drugs discovery is an urgent need. In this study, were evaluated *in vitro* the cytotoxic on human hepatocarcinoma (HepG2) and normal cells (Chang liver), and the schistosomicidal properties of crude extract, fractions and isolated compounds (1-Hydroxy-3-methoxy-*N*-methylacridone (**1**) described in this species from Cameroon for the first time, Scoparone (**2**), and Arborinine (**3**)) from powdered fruits of *Zanthoxylum leprieurii* (Rutaceae). All fractions: hexanic (FH), methylene chloride (FC), ethyl acetate (FA) and methanolic (FM) killed all the cercariae within 2 hours exposure and presents LC_{50} values between 2 and 3 µg/ml; Compounds **1** and **3** also displayed a good *in vitro* schistosomicidal activity against cercariae with LC_{50} values of 78.78 and 6.98 µg/mL, respectively. For antitumor activity compounds **1**-**3** and fraction FC presents good activity with IC_{50} values range 18.27 - 74.61 µg/mL on HepG2 cells, however most of these were more toxic on Chang cells than to HepG2 cells, with only exception for compound **2**. The acridone Arborinine (**3**) can constitute a good lead for the research of schistosomiasis alternative therapy, and the coumarin Scoparone (**2**) can be used in drug design as scaffold for design new potential anticancer agents.

Keywords: Acridone Alkaloids; Arborinine; Scoparone; Cercariae; Cytotoxic activity, Schistosomicidal activity; *Zanthoxylum leprieurii*.

1. Introduction

Schistosomiasis is a major and chronic Neglected Tropical Disease (NTD), caused by flatworm parasites belonging to the genus *Schistosoma*, predominantly S*chistosoma mansoni* (*S. mansoni*), *Schistosoma haematobium* and *Schistosoma japonicum* [1]. It is reported in 80 countries [2], affecting more than 230 million people worldwide [3] and close to 800 million are in risk, being the African countries responsible for 85% of all schistosomiasis cases [4]. It mainly affects the social and economic progress of many developing countries [5], which cannot afford to pay the high costs of research and drug development.

Among the fighting strategies, Sm- p80-Based schistosomiasis vaccine development known as Bihvax ® [6], modeling molecular agents [2] are in pipeline and they are a long process, until they can be used by the most affected population. Furthermore, although Mass Drug Administration (MDA) for schistosomiasis programs is effective in reducing the prevalence of infection in high-prevalence areas, it is much less effective in low-prevalence areas [7, 8].

The general approach for the treatment and control of morbidity through periodic treatment of all forms of schistosomiasis is the use of Praziquantel (PZQ) (**4**) commercially known as Biltricide ®; this compound was developed for adult worms in the 1970s and it is distributed to millions of people every year [9]. Unfortunately, PZQ does not kill immature schistosomes; It is rapidly metabolized and cannot prevent reinfections [2]. Although Praziquantel-based control programs was introduced to reduce the cost of PZQ and consequently for

schistosomiasis control [10, 11], this have a temporary effect on transmission and it is limited in his potential to interrupt disease transmission or reinfection in the long term; It is the evidence for a severe rebound morbidity when chemotherapy campaigns are interrupted [4]. In addition, it is inadequate for treating young children [2]. Despite the clinical relevant resistance of PZQ which has not yet been observed, reduced susceptibility in field isolates of *S. mansoni* have been found in different localities [2, 12]. All these consideration along with reliance on a single drug have highlighted the major need for novel drugs to treat schistosomiasis from the larva stage of a parasite to the adult schistosomes, as well as young children [7, 13].

Artemisinin (**5**) (Artenam ®) has shown activity against the intra-mammalian larval stage of *S. mansoni*- the schistosomula- and a liver stage *in vivo* [14, 15]; but it is less efficacious than PZQ [16] and its application to control schistosomiasis could have serious adverse side effect of increasing Artemisinin drug resistance in malaria [17].

Nonetheless, an ideal natural schistosomicidal lead candidate has yet to be identified; much research is required to overcome the limitation of current schistosomicidal drugs and the appearance of resistant schistosome strains. In view of this alarming situation, efforts should be made to identify new lead structures, effective and safe drugs, and the nature has been demonstrated to be a good source of lead compounds for drug discovery for neglected tropical diseases.

Previous works reported the *in vitro* assessment of the plant extracts against two life's stages of *Schistosoma mansoni* [18, 19]. Although only a small proportion of existing natural compounds have been evaluated against the adult worms stage [20-24], many compounds have not yet being investigated against the larva stage of *S. mansoni*. The Cameroonian folk medicine contains the use of *Zanthoxylum leprieurii* (Rutaceae) in the treatment of sterility and Sexual Transmitted Diseases (STD) [25]. This aim of the present study is evaluated new natural lead candidates from *Z. leprieurii* that may be used to develop new safe and effective drugs against schistosomiasis and human cancer.

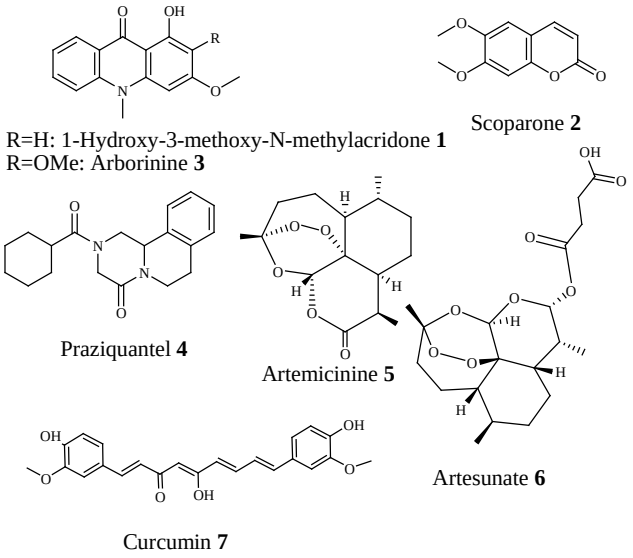

Figure 1: Chemical structures of **1**-**3** from *Z. leprieurii* and **4**-**7** for reference drugs

The phytochemical screening of crude extract and derived fractions of *Z. leprieurii* revealed the presence of alkaloids, triterpenes and coumarins. A previous antiparasitic studies in *Zanthoxylum* genus had revealed that some acridone alkaloids displayed significant antiplasmodial against *Plasmodium falciparum* [26] and antifungal activities [27]. Based on the phytochemical tests, chromatograms and test profile of extracts and fractions, FM was then subjected to several chromatography on normal silica gel column chromatography. To the best of our knowledge, no study with *Z. leprieurii* against *S. mansoni* larva stage (cercariae) have been performed to date; this encouraged us to pursue the chemical investigations of this plant in parallel with biological activities. The spectral data of all the isolated compounds were in agreement with previously published data, which allowed the identification of two acridone alkaloids , 1- hydroxy-3-methoxy-*N*-methylacridone (**1**) and Arborinine (**3**) [26, 28-31] and the coumarin, Scoparone (**2**) [28, 31-32]. Previous phytochemical studies on the seed of this plant and various Rutaceae species have described the isolation of acridone alkaloids and coumarins [26,28, 33]. Meanwhile compound **1** have been reported for *Zanthoxylum leprieurii* from Nigeria by Fish and Waterman [34] and previously from Bunalema and his colleagues [35]; but, to the best of our knowledge, this is the first report of compound **1** from *Zanthoxylum leprieurii* species from Cameroon. All isolated compounds, crude extract and fractions of *Z. leprieurii* were tested against cercariae of *S. mansoni*, as well as against two cell lines-cancer liver cell lines HepG2 (human hepatocarcinoma) and Chang liver (normal liver) cell lines.

2. Results and Discussion

The cercaricidal activity was evaluated against the larvae stage of *S. mansoni* (cercariae). The kinetics of mortality of the cercariae of *S. mansoni* after exposure to the crude extract, fractions and the compounds from *Z. leprieurii* are presented in Figure 2: A-H. In Table 1 is shown the cercaricidal activity, after 2h of exposure, expressed by their LC_{50} values (the concentration of substance necessary to kill 50 % of the animals in a batch).

In general, the results indicates that cercariaes death occurs in a dose-dependent manner for all extracts. The beginning of death begins after 15 min. of exposition of the cercariae (Figure 2: D-H). With the concentration of 100 µg/ml of the FH, all the cercariae died (Figure 2H). On the other hand, one notes a death rate of 100% after 30 min. when cercariae are exposed to the crude extract (FE) (Figure 2E), methylene chloride (FC) (Figure 2F), and methanol fractions (FM) (Figure 2D) at 100, 50 and 25 µg/ml. In low concentrations (25, 12.5, and 6.25 µg/ml) some cercariae survived after respective time's intervals of 90 and 120 min. respectively (Figures 2: D-H). An increase in the mortality rate of the cercariae for 15, 30, 60, 90, 120 and 150 min. (Figures 2: A-C). Compound **3** caused 100% cercariae death after 60 min. at concentration less than 50 µg/ml, while **1** although was more active than **2**, however none of them caused 100 % death. Comparing the effect of different concentrations of the crude extract, fractions and isolated compounds (**1**, **2**, **3**) on the *S. mansoni* cercariae mortality clearly shows that **3** (Figure 2C) is the most active compound, but less active than all the fractions. This might strongly indicate and justify synergistic effects of the chemical constituent's presents in these fractions. Arborinine **3** is the most active component, making *Z. leprieurii* a potential source of drugs for the fight against schistosomiasis. Considering the cercaricidal assay, the exposure of the *S. mansoni* cercariae to crude extract, fractions and compounds **1-3** showed that the plant have larvicidal activities against *S. mansoni*

larvae (Table 1). All fractions (FH, FC, FA, FM) and compound **3** showed strong cercaricidal potency (LC_{50} < 5 µg/ml for fraction and LC_{50} = 6.98 µg/ml for compound **3**). Crude extract and compound **1** present low activities (LC_{50} < 20-100 µg/ml), while compound **2** is considered inactive (LC_{50} > 100 µg/ml). None of cercariae in the control group died. The considerable toxic effect of plant *Z. leprieurii* might be assigned to the phytochemicals such as Acridone alkaloids (compounds **1** and **3**) due to their ability to form complexes with cholesterol and decrease its level in plasma and increase cholinesterase activity or may be decrease the frequency of cardiac contraction [36].

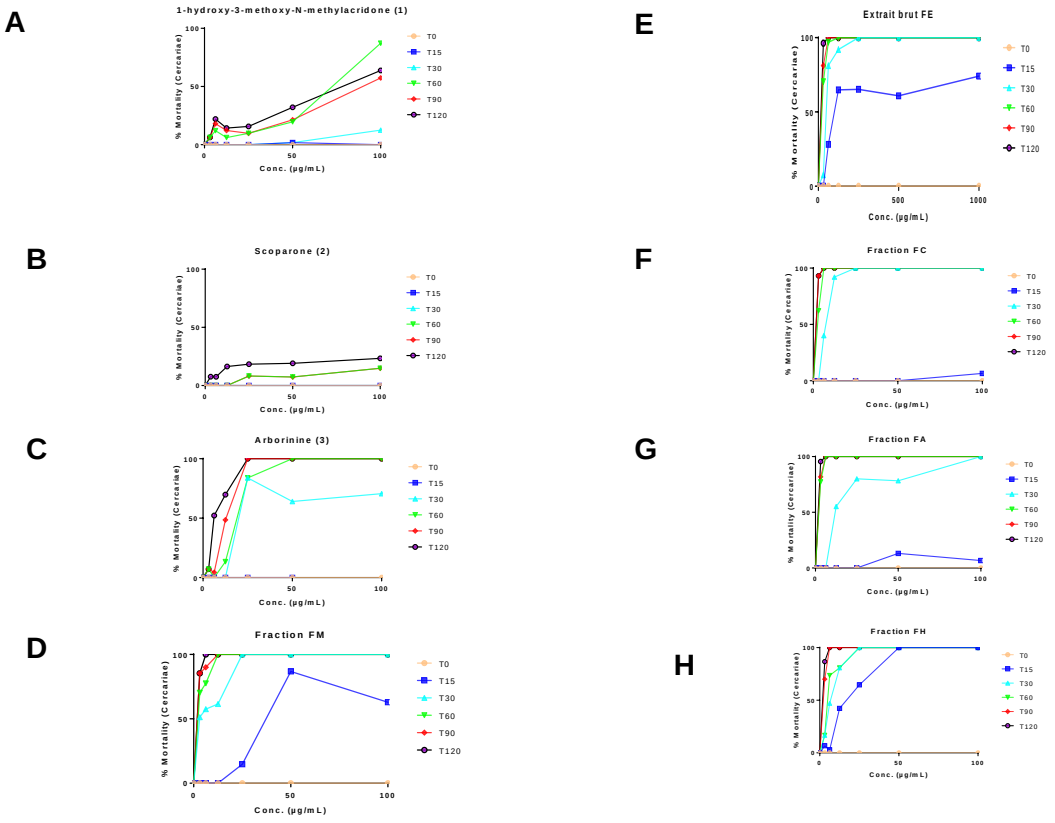

Figure 2: Effect of different concentrations of crude extract, fractions, and isolated compounds from *Z. lepieurii*, on the mortality of *S. mansoni* cercariae; **A**: Compound **1**; B: Compound **2**; **C**: Compound **3**; **D**: Fraction FM; **E**: Crude extract (FE); **F**: Fraction FC; **G**: Fraction FA; **H**: Fraction FH;

Cancer is associated with another species of *Schistosoma* (*Schistosoma haematobium*); so, it is also more prudent and essential to investigate together novel anti-cancer and antiparasitic drugs with less chance of the development of resistance against them. This corroborate with research done by McGaw and his colleagues [37] in which they mentioned the need of cytotoxicity evaluation. They showed that cytotoxicidal, or cell killing effect is required to demonstrate efficacy of an anticancer agent, though verifying a lack of toxicity of a pharmaceutical substance is far more complicated and may require analysis of specific targets such as alteration of gene transcription, cell signaling, or cell-cell interactions and usually involving many animal experiments.

In addition, research done by Hotez and his colleagues [38] on the interpretation and implications for the neglected tropical diseases showed that there has been a shift away on Daly composition during the period

1990–2010 from communicable (infectious diseases including parasitic diseases) to non-communicable diseases (NCD) (cardiovascular disease, stroke, cancer especially liver and pancreatic malignancies). Nonetheless, some of these NCD could be originated in infectious diseases including parasitic infections [38]. Additionally, a systematic review have been done by Machicado and Marcos [39] on Carcinogenesis associated with parasites other than *Schistosoma, Opisthorchis* and *Clonorchis*, they have found that parasites could potentially be associated with cancers or tumors but further evidence is needed to elaborate a cause-effect relationship. In this study, the *in vitro* cytotoxicity of extract, fractions, and isolated compounds **1-3** were evaluated at different concentrations (62.5–1000 µg/mL) against Human hepatocarcinoma (HepG2) and Chang liver cells lines, after 72h. Table 1 presents the results of the cytotoxicity evaluation in IC_{50} values (concentration to reduce growth of cancer cells by 50%) and the selectivity index (SI). Curcumin was used as reference drug.

Table 1: Anti-cercariae activity (LC_{50}), cytotoxic effects on Chang liver and

Samples	**Cytotoxicity**	**Anti-cercariae activity**		**Antitumor activity**	
	Chang liver			**HepG2**	
	IC_{50}	**LC_{50}**	**SI**	**IC_{50}**	**SI**
FE	>500	25.62	> 19.51	173.57	> 2.88
FH	146.95	2.94	49.98	270.91	0.54
FC	20.64	2.76	7.47	74.61	0.27
FA	155.87	2.73	57.09	107.23	1.45
FM	21.74	2.96	7.34	372.13	0.05
1	6.53	78.78	0.08	18.27	0.35
2	116.25	1365	0.08	44.93	2.58
3	7.62	6.98	1.09	59.17	0.12
Curcumin	6.68	nd	nd	8.30	0.80

nd: not determinated.

SI (Selective index) = IC_{50} on Chang cell / LC_{50} or IC_{50} on HepG2.

HepG2 cells (IC_{50}), and selective index values for crude extract, fractions and compounds **1**-**3** from *Z. leprieurii,* and curcumin. Values in µg/mL

The cytotoxicity data was found to be dose dependent and increases with increased concentrations. The present results is concentration are in agreement with those obtained by Tekwu and his colleagues [18] who reported the cercaricidal activities of stem bark and roots of *Rauwolfia vomitoria* as both time and concentration dependent. As reported by Wibowo and his colleagues [40], sample that IC50 inhibition < 5 µg/mL has a very strong activity; whether IC50 < 5–10 µg/mL, it has a strong activity; and a moderate activity when IC50 < 20 µg/mL; if his activity is weak IC50 < 20–100 µg/ml and not toxic when IC50 > 100 µg/mL in cell line assays.

Based on this criteria FE, FH, FA were not toxic against both cell lines. Compound **2** was not toxic only for Chang liver cell line (IC_{50} = 116.25 µg/mL). FC fraction, compounds **1** and **3** were toxic in both cells. Compounds **1** and **3**, FH, FC and FM were more toxic against Chang liver cells than HepG2 cells, indicating that they are inappropriate for use as antitumor agents. Compound **2** and FE fractions are the only that present a safety margin for conducting *in vivo* assays, since it presents SI greater than 2 (two).

Interest of effects of bioactive compounds on cancer treatment and prevention has increased dramatically over the past twenty years [41]. Such compounds have been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells [42]. Hepatocellular carcinoma is the third most common cause of cancer-related deaths worldwide [43], and about half a million individuals die from this disease annually [44].

Curcumin or diferuloylmethanol (**7**) used as drug reference is a yellow spice that is utilized in curry. Research has been established that curcumin appears both as e preventive and therapeutic agent able to reverse, inhibit or prevent the development of cancer by inhibiting specific molecular signaling pathways involved in carcinogenesis *in vitro, in vivo* and in clinical studies [45]. Deplorably, the translation of this natural product isolated from *Curcuma longa* into clinical application has been hampered due to his poor pharmacodynamics properties (poor solubility in aqueous medium and short biological half-life, link to his rapid metabolism and elimination by liver). Thus, it is imperative to search for new alternative to human cancer prevention and treatment agents.

3. Conclusion

Based on the *in vitro* evaluation, compounds **1** and **3**, and especially fractions FH, FC, FA, FM and crude extract FE were very active against cercariae. The fractions are safe as they are poorly toxic or non-toxic to normal human cells, and may have their activities explored and deepened, making it necessary to identify new substances, aiming at identifying the main components related to this activity. Nonetheless, further investigation against juvenile, preadult and adult worm stages might be useful. For anticancer activity, only compound **2** and the crude extract (FE) presented safety margin, being compound **2**, one of the most active, and selectively

cytotoxic against Hepatocarcinoma HepG2 cell, even though this cytotoxicity activity is weak, and the structural modification of this compound could increase the cytotoxicity activities of derivative will promised potential Hepatocellular carcinoma candidate cancer drugs.

4. Experimental

Reagents: Hexane, Ethyle acetate, Methanol, dichloromethane were purchased from LOUIS DREYFUS Commodities Yaounde-Cameroon; Ethanol, 3-(4,5-dimethylthiazol-2-yl)-5 diphenyltetrazoliumbromide (MTT), fetal bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbeccos Modified Eagle's Medium (DMEM), antibiotics (penicillin), were obtained from SIGMA Aldrich Co USA.

General experimental procedures: Nuclear magnetic resonance (NMR) spectra were recorded using either Bruker 400MHz or Bruker DRX500 MHz. These spectra were recorded at ambient temperature in deutered chloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ and coupling constants (*J*) in Hz. LC-MS spectra were recorded on a VG Autospec Mass Spectrometer at 70eV. The retention time and fragments were described as a relation between atomic mass units and charge (*m/z*) and the relative abundance in percentage of the base peak intensity. Infrared spectra were recorded as thin films between KBr plates for oils and as KBr discs for solid using a Perkin–Elmer 1720-X Fourier Transform Spectrometer. Melting points were recorded on a on a Buchi type apparatus, determined in a capillary and were uncorrected. Column chromatography (CC) was carried out on columns prepared as slurries of Merck silica gel 60 (70-230 mesh) in the eluent (a mixture of hexane and ethyl acetate. Preadsorption was carried out on Merck silica gel 60 (0,063-0.2 mm / 70-230 mesh). Thin layer chromatography (TLC) was carried out on silica gel plates precoated with 60 F_{254} from Sigma Aldrich using a mixture of hexane and ethyl acetate as eluent. The visualization of the compounds on the chromatograms was made under Ultraviolet light (UV: √=254; 365nm) and spray with 50 % diluted sulfuric acid in water, followed by mild heating.

Plant Materials: Fruits of *Z. leprieurii* (locally called «Melam» in Nguemba dialect) were collected in August 2014 from Bamougoum, a District of Bafoussam III, in the West region of Cameroun. A sample of the young plant (three years old) was deposited at the National Herbarum Cameroun (NHC) and identified with the help of a botanist (Dr Jean Michel Onana from the NHC) with the collector number NKWENGOUA ZE1 of the specimen of the NHC collection under the number 66931 Cam (YA). Fresh fruits were rinsed thoroughly in running tap water and dried at room temperature (~27.9 °C), powdered and stored in an air-tight container for phytochemical and biological tests.

Extraction, Fractionation of the Plant Materials, Isolation and purification of compounds: The powder (3200 g) obtained after the pulverization of the plant material was soaked in the mixture of methylene chloride (3000 mL) and methanol (3000 mL) for 72 hours with temporal shaking. The filtrate was concentrated under reduced pressure using a rotary evaporator to give the crude extract (FE, 705.3 g). FE was then subjected to successive fractionation by watching it successively with hexane (FH 1000 mL), methylene chloride (FC, 2000 mL), ethyl acetate (FA, 2000 mL) and methanol FM (4500 mL). This fractionation afforded 42.2 g of FH fraction (FH), 73.8 g of FC fraction (FC), 116.4 g of FA fraction (FA) and 472.7 g of FM fraction (FM). These extract and

fractions were then stored at −20˚C until further use. Based on the phytochemical tests and chromatographic profile of extracts and fractions, FM (60 g) was then subjected to several chromatography on normal silica gel column chromatography (CC: 0.063-0.2 mm / 70-230 mesh from Merck; 4 cm × 60 cm; 200 g) and eluted with increasing quantities of ethyl acetate in hexane by increasing polarity. Three compounds were obtained: 1-Hydroxy-3-methoxy-*N*-methylacridone (**1**) (47 mg; mp: 206.09 ^{O}C) **[**28a**]** was obtained from fractions 63-79 (0.950 g) by recrystallization in the mixture of solvent hexane/ethyl acetate 85/15; Scoparone (**2**) (73.8 mg; m/z: no ionization by LC-MS cESI; 143.9 $^{\circ}$C) [26, 28b-31] was obtained from fractions 101-112 (1.02 g) by recrystallization in hexane/ethyl acetate 70/30; and Arborinine (**3)** (116.4 mg; m/z: 286.15 g/mol; 175.5 $^{\circ}$C) [28b, 31] was obtained from fractions 113-135 (1.9 g) by recrystallization in hexane/ethyl acetate 65/35;

Spectral data of isolated compounds: All compounds (**1-3**) have already been described in the literature [28-30] and the spectroscopic data are in accordance with previously related data.

Phytochemical screening: A chemical screening in plant extract FE was carried out according to the methods described by Bruneton and his colleagues [46] with an aim of evaluating the various classes of secondary metabolites that contain these young fruits. The phytochemical screening of the isolated compound allows us to confirm the class to which the compounds belong.

In vitro Studies with S. mansoni

Stock and Working Plant Extract Solutions: *In vitro* schistosomicidal evaluation was conducted on cercariae evolutionary form. Stock solutions of extract, fractions and isolated compounds were prepared in dimethylsulfoxide (DMSO, Merck) aliquoted and kept at -20°C until used. Working solutions were freshly prepared for bioassay. The maximum final concentration of DMSO in all assays was ≤1% and this was used as a solvent control. An African strain of S. *mansoni,* from Ghana, was maintained in the laboratory using *Biomphalaria pfeifferi*s nails. The snail *B. pfeifferi,* which are the schistosomiasis intermediate host snails for *S. mansoni,* were collected from endemic areas in their natural habitats from Tomefa along the Weija River in Ghana. The snails were transported to the Snail Laboratory at the Department of Parasitology, Noguchi Memorial Institute for Medical Research (NMIMR), for maintenance. They were examined for cercariae shedding and kept in aquarium containing dechlorinated tap water at room temperature (25°C).

Preparation of Cercarial Suspension*:* Schistosome cercariae's were obtained from experimentally infected *B. pfeifferi*s nails as described previously [18].

In vitro Cercariacidal Activity Test: The effects of crude extract (FE), fractions (FH, FC, FA FM) and isolated compounds **1-3** on *Schistosoma* infectious stage (cercariae) were assessed as previously described by Tekwu and his colleagues [18]. Briefly, series of crude extract (FE), and fractions (FH, FC, FA FM) at concentrations of 31.25, 62.5, 125, 250, 500, and 1000 μg/mL, and compounds **1-3** at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL were freshly prepared in a 24-microtiter well plate (Costar) and analyzed alongside with the positive control (Artesunate (**6**), 10 μg/mL). An average number of 20 freshly shed cercariae were transferred

into each well plate (Costar) using micropipette. The same number of cercariae was placed in a well containing 1% DMSO as negative control. All experiments were carried out in duplicate and were repeated. Mobility and viability of the cercariae were observed for 2 h at 30 min intervals since infectivity of cercariae is known to be rapidly lost after 12 h. Unaffected free swimming larvae, immobile, and dead cercariae at the bottom of the wells were observed at 4x magnification with an inverted microscope (Olympus CK 300). Survival and mortality at a successive interval of 15, 30, 60, 90, 120, and 150 min. were recorded. Cercariae were presumed dead when they stopped movement and sank down and their tail were detached. The LC_{50} values of the crude extract (FE), fractions (FH, FC, FA FM) on schistosome cercariae were determined at 1h and 2h. ***Cytotoxicity of the Prepared Crude Extract, fractions and isolated compounds 1-3*:** The crude extract (FE), fractions (FH, FC, FA, FM) and isolated compounds **1**-**3** were evaluated in concentration response (CR) assays against two cell lines-cancer liver cell lines HepG2 (human hepatocarcinoma) and Chang liver (normal liver) cell lines as described in our previous publication [18]. Briefly, stock cells HepG2 and Chang liver were, cultured in Dulbeccos Modified Eagle's Medium (DMEM) and Roswell Park Memorial institute (RPMI) 1640 respectively. Each medium was supplemented with 10% FBS and 1% penicillin-streptomycin and cultures were then incubated at 37°C under 5% CO2 in fully humidified conditions until 80% confluence. The stock cultures were grown in 25 cm2 culture flasks and cells were detached from the surface of the culture flask with 0.25% trypsin solution. All experiments were carried out in flat-bottom 96-well microtiter plates (Corning Incorporated, USA). Fresh stock solutions of the plant extracts, fractions and compounds were made up with DMEM (for HepG2 cell) and RPMI1640 (for Chang liver cell) supplemented with 10% FBS and 1% penicillin streptomycin to obtain a concentration of 10 µ g/mL and sterilized by filtration. Serial two fold dilutions were prepared from the stock. The monolayer cell culture was trypsinized and cell count was adjusted to 10^5 cells/mL using DMEM or RPMI1640 accordingly containing 10% FBS and 1% penicillin-streptomycin. To each well of the 96-well microtiter plates, 100 µL of the diluted cell suspension at a density of approximately 100,000 cells was plated. After 24h of incubation, when a partial monolayer was formed, cells were treated for another 72h with various concentrations of each of the plant extracts and curcumin (**7**) as positive control. Subsequently, 20 µL of 2.5 mg/mL MTT in PBS was added to each well and the cells were incubated for another 4h. The precipitated MTT formazan product was dissolved in 100 of 0.04 N HCl isopropanol in the dark and at room temperature overnight. The amount of formazan formed was measured at a wavelength of 570 nm using a microplate reader (TECAN Infinite M200 Pro Plate Reader, Austria). Cytotoxicity was calculated as the percentage of live cells relative to the control culture using the following formula:

$$\%\ cell\ viability\ (CV) = (100\ x\ Absorbance\ of\ the\ cell - Absorbance\ of\ drug\ color\ control) \div (absorbance\ of\ untreated\ cell - Absorbance\ of\ black)$$

The concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

Selectivity Index (SI): In the present study, the degree of selectivity of each ethanol plant extract and/or isolated compound is expressed as the ratio of the IC_{50} to the LC_{50} for *S. mansoni* cercariae or IC50 in cancer

cell line.

$$Selectivity\ Index\ (SI) = IC50\ of\ sample\ in\ cell\ lines\ \div\ LC50\ of\ the\ same\ sample\ in\ S.mansoni\ cercariae\ or\ IC50\ in\ cancer\ cell\ line$$

Statistical Analysis: Graph drawing and statistical were performed analysis using GraphPad Software (version 7.00). The data were expressed as means ± SD, and Student's *t-test* was used to determine significance of differences between mean values. *P* values less than 0.05 were considered statistically significant.

Conflict of interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

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